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(54) Title: BENZOATE BUFFERS FOR ZONE ELECTROPHORESIS AND IMMUNOFIXATION

(57) Abstract: Buffers comprising benzoic acid and/or a salt thereof are disclosed, which buffers are suitably for use for zone electrophoresis and/or immunofixation. Furthermore, the use of benzoic acid and/or a salt thereof for zone electrophoresis and/or immunofixation is disclosed. Kits for zone electrophoresis and/or immunofixation are also provided.

BENZOATE BUFFERS FOR ZONE ELECTROPHORESIS AND IMMUNOFIXATION

The present invention relates to buffers comprising benzoic acid and/or a salt thereof for use in zone electrophoresis and/or immunofixation. The invention further relates to the use of benzoic acid and/or a salt thereof as buffer components for zone electrophoresis and/or immunofixation. The invention also concerns kits for zone electrophoresis and/or immunofixation. An optimised combination of the gel buffer and the compartment buffer is disclosed herein giving sharper and easier identifiable protein bands or protein pattern. A special advantage being that the buffers disclosed herein are non-hazardous.

BACKGROUND OF THE INVENTION

The principle of immunofixation was described by Alfonzo and Wilson in 1964 (ref. 1). The method was later modified by Alper and Johnson in 1969 and used for identification of genetic protein variants (ref. 2). Immunofixation is a widely used diagnostic method. It is a rapid, important and useful tool for the examination and identification of various protein abnormalities in serum, urine, cerebrospinal and synovial fluids.

The immunofixation procedure can be used for the identification of any single protein band of an electrophoresis. The technique is a combination of zone electrophoresis followed by immunofixation using monospecific antibodies. In this way it is possible to separate and identify different proteins in a biological mixture according to their physicochemical properties and antigenic properties.

The immunofixation procedure is most frequently used for the detection of monoclonal immunoglobulins in serum and Bence Jones proteins in urine.

Usually, barbital buffers comprising barbituric acid and/or sodium barbiturate are used (ref. 3). In fact, this use is recommended as barbituric acid/sodium barbiturate provide a good separation of all protein bands. However, barbituric acid and sodium barbiturate are hazardous compounds which potentially cause irritation by contact with the skin, the eyes or the respiratory system, and which in extreme cases even may cause death. In more and more countries, the use of barbituric acid and sodium barbiturate in buffers is therefore prohibited.

Thus, buffer components which can replace barbituric acid and sodium barbiturate and which further possess the advantages of barbituric acid and/or sodium barbiturate are still needed. The present invention provides such compounds which can replace barbituric acid and sodium barbiturate.

SUMMARY OF THE INVENTION

In a first aspect, the present invention relates to buffers comprising benzoic acid and/or a salt thereof for zone electrophoresis and/or immunofixation.

In another aspect, the present invention relates to the use of benzoic acid and/or a salt thereof as buffer components for zone electrophoresis and/or immunofixation.

In a third aspect, the present invention concerns kits for zone electrophoresis and/or immunofixation comprising a buffer as described herein.

The present invention is described in detail in the following.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 shows an immunofixation gel obtained following the procedures described in Example 1.

Figures 2A, 2B, 2C and 2D show four patient serum samples subjected to zone electrophoresis and immunofixation with the buffer of the invention comprising the sodium salt of benzoic acid.

Figures 3A, 3B, 3C and 3D show the four patient serum samples as shown in Figures 2A, 2B, 2C and 2D subjected to zone electrophoresis and immunofixation with the buffer of the invention comprising the ammonium salt of benzoic acid.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates buffers for use in zone electrophoresis and/or immunofixation comprising benzoic acid and/or a salt thereof.

From Chromatographia Vol. 48, No. 5/6, 383-387 (1998) (ref. 4), Deutsche Lebensmittel-Rundschau 94. Jahrg., Heft 1, 28-30 (1998) (ref. 5), Commun. Soil Sci. Plant Anal. 30 (1 & 2), 213-220 (1999) (ref. 6), Journal of Chromatography A. 781, 497-501 (1997) (ref. 7), Journal of Pharmaceutical and Biomedical Analysis 15, 63-71 (1996) (ref. 8), and J. Cap. Elec. Vol. 2, No. 5, 235-240

(1995) (ref. 9) buffers containing benzoic acid or sodium benzoate are known. The buffers are used in capillary electrophoresis for the determination of i.a. organic acids, phosphate, phytin acid, short-chain fatty acids, and cyclodextrins. The principle of capillary electrophoresis is very different from the principles of zone electrophoresis in gels and immunofixation.

From US 4,321,119 (ref. 10), a non-barbiturate buffer composition for use in the electrophoretic separation of proteins into fractions is known, said buffer comprising a water soluble salicylate such as sodium salicylate and an inorganic salt. The salicylate is used in a concentration of from 0.5 to 1 g salicylate to 1.5 to 3 g inorganic salt.

In particular, the buffer of the invention may comprise a salt of benzoic acid. Examples of suitable salts is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt of benzoic acid. The buffer of the invention may comprise one or more of these salts of benzoic acid optionally in combination with benzoic acid itself. Accordingly, benzoic acid or one or more salts of benzoic acid may be used alone, or benzoic acid and one or more salts of benzoic acid may be used in combination. In a preferred embodiment, the buffer of the invention comprises a salt of benzoic acid, in particular the sodium salt of benzoic acid.

The buffer of the invention is particularly suitable for separation of proteins, serum proteins, and immunoglobulins in zone electrophoresis and/or immunofixation. Proteins are large molecules which are susceptible to denaturation, thus, making them sensitive to the conditions employed in the separation procedure. E.g. heat development during the electrophoresis

procedure may result in destruction of the protein structure. Increased ionic strength of the running buffer increases the electric conductivity, thus leading to an increased heat development. This can be avoided e.g. by using heavy cooling procedures during the electrophoresis procedure. Alternatively, low concentration of ion-providing components of the running buffer can be used. For instance, in US 4,321,119, a relatively low concentration of salicylate is used.

However, it has surprisingly been found that a buffer comprising benzoic acid and/or a salt thereof does not suffer from the above drawbacks. The benzoic acid and the salts thereof can be used in relatively high concentrations without the need for additional cooling in order to avoid breakdown of the protein structure or denaturation. An additional advantageous feature of the buffer is that the benzoic acid and the salts thereof in general act as preserving agents. This is especially observed in the case of sodium benzoate (the sodium salt of benzoic acid).

The buffer of the invention is used as a gel buffer and/or as a compartment buffer. The term "buffer" includes both. The gel buffer and the compartment buffer may have the same acid/salt concentration, or the concentrations of the gel buffer and the compartment buffer may be different.

The gel buffer suitably comprises benzoic acid and/or a salt thereof in a concentration of from 1 to 10 g/L. In a preferred embodiment, the buffer comprises benzoic acid and/or a salt thereof in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L. In particular, the concentration of benzoic

acid and/or the salt thereof may be 7 g/L. Surprisingly, it seems as if the concentration range yielding a good separation is peak-like, having a maximum in the concentration range from 6.5 to 7.5 g/L, more specifically around a concentration of about 7 g/L.

The compartment buffer suitably comprises benzoic acid and/or a salt thereof in a concentration of from 1 to 10 g/L. In a preferred embodiment, the buffer comprises benzoic acid and/or a salt thereof in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L. In particular, the concentration of benzoic acid and/or the salt thereof may be 3.5 g/L. Surprisingly, it seems as if the concentration range yielding a good separation is peak-like, having a maximum in the concentration range 3 to 4 g/L, more specifically around a concentration of about 3.5 g/L.

It lies within the scope of the present invention to use each of the components (benzoic acid itself or any of its salts) in the concentrations specified above.

The zone electrophoresis/immunofixation procedure is a very powerful tool in the early diagnosis of various diseases. Frequently, the disease can be diagnosed even before the patient experiences symptoms of the disease. Therefore, a distinct separation of the protein bands is of crucial importance in order to make a reliable diagnosis. Especially the separation of the immunoglobulins is of major importance. These proteins are produced in the bone marrow and their appearance in immunofixation reflects the status of the bone marrow. A cancer disease in the bone marrow affecting the plasma cells may lead to changed synthesis of the immunoglobulins and thereby the appearance of these

immunoglobulins in immunofixation. The normal heterogeneous pattern is most frequently changed into a pattern of distinct bands with different mobility. In particular in such cancers an early treatment regime is important for the survival of the patient and also the treatment, e.g. chemotherapy, radiation and/or plasmapheresis, as such influences the patient's well-being to a major extent. As evident from the Figures, the buffers of the invention provide such good and distinct separation enabling a reliable diagnosis.

The buffer of the invention may further comprise additional components such as Tris and/or Tricine and/or calcium lactate and/or sodium azide.

The benzoic acid compounds as defined above are suitable as buffer components, in particular as gel buffers and compartment buffers. They are much less hazardous than the conventionally used barbiturates. Furthermore, no hazard labelling of the buffers is required. It has further been shown (cf. Example 1) that the buffers of the invention seem to provide sharper and more well defined bands than the conventionally used barbiturate-containing buffers. Also, the use of a compartment buffer having a lower salt/acid concentration than the gel buffer may be advantageous. This may inhibit the heat development during electrophoresis and yield sharper and more well defined bands.

Furthermore, the buffer may contain one or more additional components such as buffering agents, preserving agents, colouring agents, salts, detergent and surfactants.

In a special embodiment of the buffer of the present invention, the gel buffer comprises the sodium salt of

benzoic acid in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L. In a preferred embodiment, the concentration of the sodium salt of benzoic acid is from 6.5 to 7.5 g/L, in particular 7 g/L.

In a special embodiment of the buffer of the present invention, the compartment buffer comprises the sodium salt of benzoic acid in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L. In a preferred embodiment, the concentration of the sodium salt of benzoic acid is from 3 to 4 g/L, in particular 3.5 g/L.

In particular, the gel buffer may have a salt concentration of 7 g/L, and the compartment buffer a concentration of 3.5 g/L.

As mentioned, the buffer is for use in zone electrophoresis, and/or immunofixation. The test samples are suitably serum, urine, cerebrospinal or synovial fluids.

In another aspect, the present invention relates to the use of benzoic acid and/or a salt thereof as a buffer component for zone electrophoresis and/or immunofixation.

Benzoic acid or a salt of benzoic acid may be used alone, or benzoic acid and one or more salts of benzoic acid may be used in combination. Examples of suitable salts of benzoic acid are the sodium salt, the potassium salt, the calcium salt, the magnesium salt and the ammonium salt. In a preferred embodiment, the salt of benzoic acid is the sodium salt.

As mentioned above, it has surprisingly been found that benzoic acid or the salts thereof can be used in relatively high concentrations. Thus, in one embodiment, the present invention relates to the use of benzoic acid and/or a salt thereof, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L. In particular, in the gel buffer, benzoic acid and/or the salt thereof may be used in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L. In particular the concentration may be from 6.5 to 7.5 g/L, like 7 g/L. In particular, in the compartment buffer, benzoic acid and/or the salt thereof may be used in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L. In particular the concentration may be from 3 to 4 g/L, like 3.5 g/L.

In a particular embodiment, the sodium salt of benzoic acid in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L is used as a component of the gel buffer. In a preferred embodiment, the sodium salt of benzoic acid in a concentration of from 6.5 to 7.5 g/L, in particular a concentration of 7 g/L, is used as a component of the gel buffer.

In a particular embodiment, the sodium salt of benzoic acid in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L is used as a component of the compartment buffer. In a preferred embodiment, the sodium salt of benzoic acid in a concentration of from 3 to 4 g/L, in particular a concentration of 3.5 g/L, is used as a component of the compartment buffer.

In a further embodiment, the present invention relates to the use of benzoic acid and/or a salt thereof in a buffer for electrophoresis and/or immunofixation, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.

In third aspect, the present invention relates to kits for zone electrophoresis and/or immunofixation, which kits comprise a buffer as defined above.

In one embodiment, the kit further comprises gels containing the buffer of the invention, staining solutions, antibodies (e.g. rabbit immunoglobulins), blotters, templates, fixation and/or reagents.

The gel to be used is suitably an agarose gel. The agarose gel may suitably be provided in a ready-to-use packing containing the buffer of the invention. The buffer of the invention may thus be used as compartment buffer as well as gel buffer for supporting medias such as agarose, starch, polyacrylamide etc.

The invention is further illustrated by the following, non-limiting example.

EXAMPLES

EXAMPLE 1

Materials. Agarose Gel, 10 plates (gel buffer). Ready-to-use. Each plate is 8.3x10.2 cm and contains on a transparent, flexible plastic backing, agarose gel containing the buffer of the invention comprising sodium benzoate (i.e. sodium salt of benzoic acid) or ammonium

benzoate (i.e. the ammonium salt of benzoic acid) (1% gel, 99% buffer) preserved with sodium azide.

Concentrated Buffer. 3x75 mL (13.33 x concentrated) buffer of the invention preserved with sodium azide. The content of each of the bottles of buffer is diluted prior to use to a total volume of 1000 mL with distilled water. The diluted buffer contains sodium benzoate (3.5 g/L) or ammonium benzoate (3.5 g/L), Tris (3.6 g/L), Tricine (0.6 g/L), calcium lactate (0.75 g/L), and sodium azide (0.04 g/L).

Concentrated Staining Solution. 75 mL (4 x concentrated). Amido Black in 5% acetic acid. The Staining Solution is diluted prior to use to a total volume of 300 mL with distilled water. The concentration of Amido Black in the diluted Solution is 5 g/L.

Test Sample. Serum samples, optionally freshly drawn. 33 samples were tested.

Sample Template. 10 pieces.

Antibody Template. 10 pieces.

Gel Blotter. Pre-cut disposable, filter paper, 1 package, 40 sheets.

Sample Blotter. Pre-cut disposable, filter paper, 1 package, 10 sheets.

Drying Blotter. Pre-cut disposable filter paper, 2 package, 20 sheets each.

Fixation Reagents. Protein Fixative Solution 1.0 mL containing 7% sulphosalicylic acid and 5% acetic acid. Green dyed.

Rabbit Anti-Human IgG. Specific for γ-chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

Rabbit Anti-Human IgA. Specific for α-chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

Rabbit Anti-Human IgM. Specific for μ-chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

Rabbit Anti-Human Kappa Light Chains. Specific for kappa light chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

Rabbit Anti-Human Lambda Light Chains. Specific for lambda light chains. Immunoglobulin fraction. 1.0 mL. Preserved with 15 mM sodium azide. Green dyed.

Other reagents. Saline Solution (0.9% NaCl). For dilution of the samples and washing of the gel. Destaining Solution (acetic acid, 5%). Distilled or deionised water.

Equipment. Power supply 120 V constant. Electrophoresis apparatus for Agarose Gels (DAKO Electrophoresis Apparatus Code No. S 2200). Pipettes (5 μL, 80 μL). Containers for washing, staining and destaining of Agarose Gels (DAKO Washing and Staining Accessory Kit Code No. S 2201). Glass plate (minimum 11×11 cm) plus a weight of approximately 1 kg for pressing the gel. Hair dryer or a drying oven (maximum 90°C).

Additional Reagents. Rabbit Anti-Human IgD (DAKO Code No. A 0093), specific for δ-chains, immunoglobulin fraction, preserved with 15 mM sodium azide. Rabbit Anti-Human IgE (DAKO Code No. A 0094), specific for ε-chains, immunoglobulin fraction, preserved with 15 mM sodium azide. Rabbit Anti-Human Kappa Free Light Chains (DAKO Code No. A 0100), specific for kappa free light chains, immunoglobulin fraction, preserved with 15 mM sodium azide. Rabbit Anti-Human Lambda Free Light Chains (DAKO Code No. A 0101), specific for lambda free light chains, immunoglobulin fraction, preserved with 15 mM sodium azide.

Preparation of specimens. All serum specimens should preferably be diluted with saline solution just prior to use. For the reference pattern, serum should be diluted 1:4 (1 part serum + 3 parts Saline Solution). For the immunofixation patterns serum should be diluted 1:15 (1 part serum + 14 parts saline solution). For serum suspected of containing low levels of monoclonal immunoglobulins, a dilution of 1:4 is recommended. For serum specimens suspected of containing high levels of monoclonal immunoglobulin (>30 g/L), a dilution of 1:31 may be suitable.

For the detection of Bence Jones proteins in urine, the urine sample should be concentrated (e.g. by ultrafiltration) to a total protein concentration of at least 1 g/L. This concentrated urine is applied in all slots. The light chain antibodies as described above will precipitate kappa or lambda chains whether they are free or still part of the immunoglobulin molecule. In order to determine if detected light chains are present as free light chains in the urine, special antibodies as

described above against free kappa and free lambda light chains could be employed.

Assay procedure

Zone Electrophoresis (separation of the proteins). All samples are prepared as described above. The Agarose Gel is removed from the foil package and placed on a level surface. Excess moisture is removed from the gel surface by gentle blotting with a Gel Blotter. The Sample Template is placed on the surface of the gel so that the slots are in alignment with the arrows located on the edges of the gel. 5 µL of the pre-diluted serum sample is applied across each slot. The 1:4 serum dilution is applied in the slot marked Ref., and the 1:15 serum dilution in the other 5 slots. The sample is allowed to diffuse into the gel for 5 minutes, and then the sample template is blotted gently with a Sample Blotter in order to remove excess sample liquid. The Blotter is discarded, and the Sample Template is carefully removed and discarded.

Electrophoresis. The DAKO Electrophoresis Apparatus is filled with 300 mL diluted buffer (150 mL in each compartment). The gel is placed in the apparatus so as to form an arch (gel side down) in such as way that the (-) side of the gel dips into the cathode compartment (-), and that the (+) side of the gel dips into the anode compartment (+). The lid is placed on the apparatus and power supply is connected. The voltage is set to 120 V and the electrophoresis is continued for 25 minutes. Upon completion of the electrophoresis, the power supply is disconnected, and the gel is carefully removed from the apparatus and placed on a level surface, gel side up. The electrophoresis buffer is discarded.

Immunofixation (specific precipitation of the separated proteins). The surface of the gel is gently blotted with a Gel Blotter. The Gel Blotter is removed immediately and discarded. The Antibody Template is placed over the surface of the gel so that the troughs of the Template are in alignment with those printed on the plastic backing of the gel. It should be ensured that a close contact between the Template and the surface of the gel is obtained. The Template is gently rubbed in order to remove air bubbles. The following is applied: Ref.: 80 µL of Protein Fixative Solution, IgG: 80 µL of Anti-IgG, IgA: 80 µL of Anti-IgA, IgM: 80 µL of Anti-IgM, K: 80 µL of Anti-Kappa, and L: 80 µL of Anti-Lambda. It should be ensured that the volume is evenly distributed within the trough. Furthermore, the surface of the gel should not be touched. The gel is incubated with the Antibody Template in a humid box for 15 minutes at room temperature. Subsequently, the gel is placed on a levelled surface and the Antibody Template is carefully removed.

Pressing, washing, staining, destaining and drying (removal of non-precipitated proteins and staining of the precipitated protein bands). The containers of the DAKO Washing and Staining Accessory Kit are filled in the following way: Washing: 1 container with 300 mL Saline Solution. Staining: 1 container with 300 mL diluted Staining Solution. Rinsing: 1 container with 300 mL distilled water. Destaining: 1 container with 300 mL acetic acid, 5%. The gel is covered with one sheet of Gel Blotter, two sheets of Drying Blotter and a glass plate. The gel is pressed under a weight of approximately 1 kg for 10 minutes. Subsequently, the Blotters are removed and discarded, and the gel is immersed in saline solution and washed for 10 minutes without agitation. The pressing procedure is repeated as previously described. After pressing, the Blotters are discarded, and the gel is

dried in a current of hot air or, alternatively, dried in a drying oven (maximum temperature 90°C) for approximately 5 minutes.

Staining is performed for 5 minutes in the Diluted Staining Solution.

Excess Staining Solution is rinsed off in distilled water before destaining. Destaining is performed in fresh Destaining Solution for approximately 2 minutes, or until the background has a faint blue colour.

Finally, the gel is dried for 5 minutes as previously described or until the gel is completely dry.

In Figures 1, 2 (A, B, C and D) and 3 (A, B, C and D) the results obtained are shown. Each immunofixation gel shows the results of one patient serum sample. The lane at the left is a reference lane showing all serum proteins in the serum sample in the order albumin (top), followed by alpha, beta, and gamma globulins (immunoglobulins). The following lanes are a visualisation of the patient's immunoglobulins established by the use of specific antibodies. From left to right, the gel shows IgG, IgA, IgM, kappa light chain, and lambda light chain.

The results shown in Figure 1 are obtained using the sodium benzoate buffer.

The results shown in Figure 2 are obtained using the sodium benzoate buffer. Figure 2A shows a patient having clearly defined double bands in the gamma region. The bands can be identified as IgM, kappa. Figure 2B shows a patient having strong double bands in the gamma region. The bands are stronger than in Figure 2A. The bands can be identified as IgM, kappa. Figure 2C shows a patient

having a strong band which can be identified as IgA, lambda. Figure 2D shows a patient having a very strong band which can be identified as IgG, lambda.

The results shown in Figure 3 are obtained using the ammonium benzoate buffer. Figures 3A, 3B, 3C and 3D shows the serum samples as in Figures 2A, 2B, 2C and 2D. The only difference being that the buffer used is an ammonium benzoate buffer. As appears from the figure, the results obtained using the ammonium benzoate buffer are not as excellent as the results obtained using the sodium benzoate buffer. However, this might be due to lack of optimisation.

Results. The overall function of the buffer is comparable to conventional barbiturate-containing buffer (DAKO Code No. K 0390). Furthermore, sharper and more well defined bands were obtained. A further advantage of the novel buffer is that it is non-hazardous.

EXAMPLE 2

A series of zone electrophoresis and immunofixation procedures using the electrophoresis/immunofixation set-up described in Example 1 were performed. The buffer used for this experiment corresponded with regard to the components to the sodium benzoate buffer described in Example 1, however, the gel buffer content of sodium benzoate was varied between 1 and 10 g/L, and the compartment buffer content of sodium benzoate was varied accordingly. The separation of the proteins was evaluated and rated 3 (excellent separation), 2 (acceptable separation) or 1 (bad separation). The results obtained are shown in the table below.

g/L sodium benzoate in gel buffer	Performance
1	1
2	1
3	1
4	2
5	2
7	3
10	1

As it appears from the table, it seems as if the performance of sodium benzoate is peak-like around a concentration maximum of about 7 g/L. Of course this peak may be shifted depending on the nature and characteristics of the other components of the buffer.

EXAMPLE 3

A series of zone electrophoresis and immunofixation procedures using the electrophoresis/immunofixation set-up described in Example 1 were performed. The buffer used for this experiment corresponded with regard to the components to the sodium benzoate buffer described in Example 1, however, the gel buffer content of sodium benzoate being 7 g/L, whereas the compartment buffer content of sodium benzoate was varied between 0.8 and 7 g/L. The separation of the proteins was evaluated and rated 3 (excellent separation), 2 (acceptable separation) or 1 (bad separation). The results obtained are shown in the table below.

g/L sodium benzoate in compartment buffer	Performance
0.8	1
1.2	1
1.8	2
3.5	3
4.7	3
7	2

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10. US 4,321,119

CLAIMS

1. Buffer for use in zone electrophoresis and/or immunofixation comprising benzoic acid and/or a salt thereof.

2. Buffer according to claim 1, comprising a salt of benzoic acid.

3. Buffer according to claim 1 or 2, comprising the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt of benzoic acid.

4. Buffer according to any one of claims 1-3, comprising the sodium salt of benzoic acid.

5. Buffer according to any one of claims 1-4, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.

6. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L in the gel buffer.

7. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L in the gel buffer.

8. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L in the compartment buffer.

9. Buffer according to claim 5, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L in the compartment buffer.

10. Buffer according to any one of claims 1-9, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.

11. Use of benzoic acid and/or a salt thereof as a buffer component for zone electrophoresis and/or immunofixation.

12. Use according to claim 11, wherein a salt of benzoic acid is used.

13. Use according to claim 11 or 12, wherein the salt of benzoic acid is the sodium salt, the potassium salt, the calcium salt, the magnesium salt and/or the ammonium salt.

14. Use according to any one of claims 11-13, wherein the salt of benzoic acid is the sodium salt.

15. Use according to any one of claims 11-14, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 10 g/L.

16. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 3 to 8 g/L, 3.5 to 8 g/L, 4 to 8 g/L, 4.5 to 8 g/L, 5 to 8 g/L, 5.5 to 8 g/L, 6 to 8 g/L, 6.5 to 8 g/L, 7 to 8 g/L, or 7.5 to 8 g/L in the gel buffer.

17. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of 7 g/L in the gel buffer.

18. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of from 1 to 5 g/L, 1.5 to 5 g/L, 2 to 5 g/L, 2.5 to 5 g/L, 3 to 5 g/L, 3.5 to 5 g/L, 4 to 5 g/L, or 4.5 to 5 g/L in the compartment buffer.

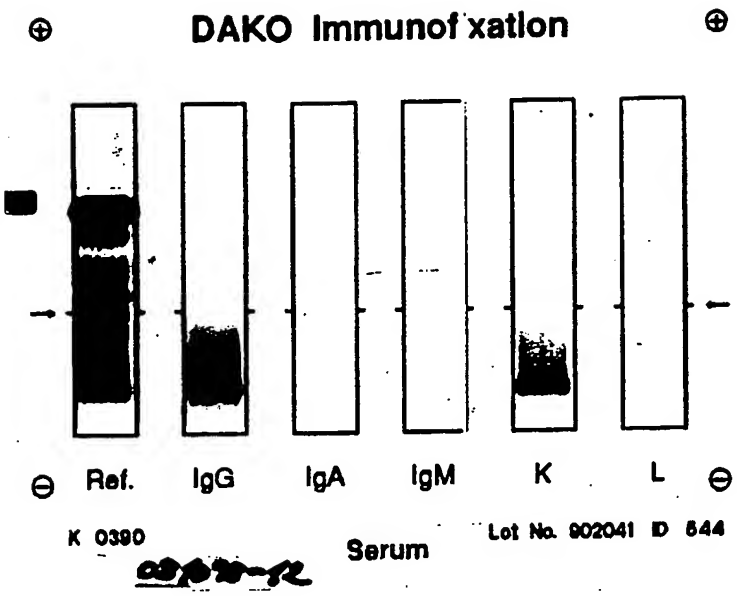
19. Use according to claim 15, wherein the benzoic acid and/or the salt thereof is present in a concentration of 3.5 g/L in the compartment buffer.

20. Use according to any one of claims 11-19, wherein the buffer further comprises Tris and/or Tricine and/or calcium lactate and/or sodium azide.

21. Kit for zone electrophoresis and/or immunofixation comprising a buffer as defined in any one of claims 1-10.

22. Kit according to claim 21 further comprising gels containing the buffer as defined in any one of claims 1-10, staining solutions, antibodies, blotters, templates, and/or fixation reagents.

Fig. 1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K 0390
Serum
Lot No. 902041 ID 644

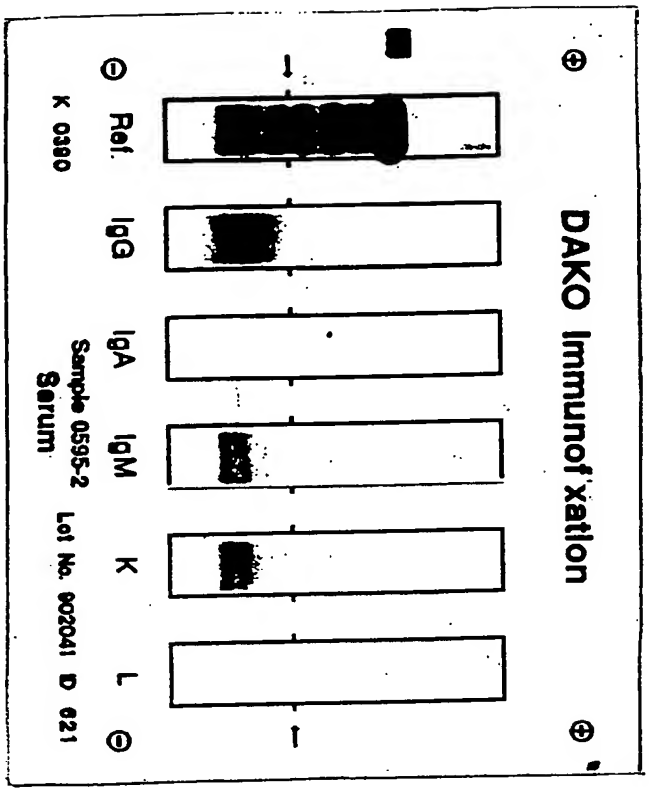
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Fig. 2A

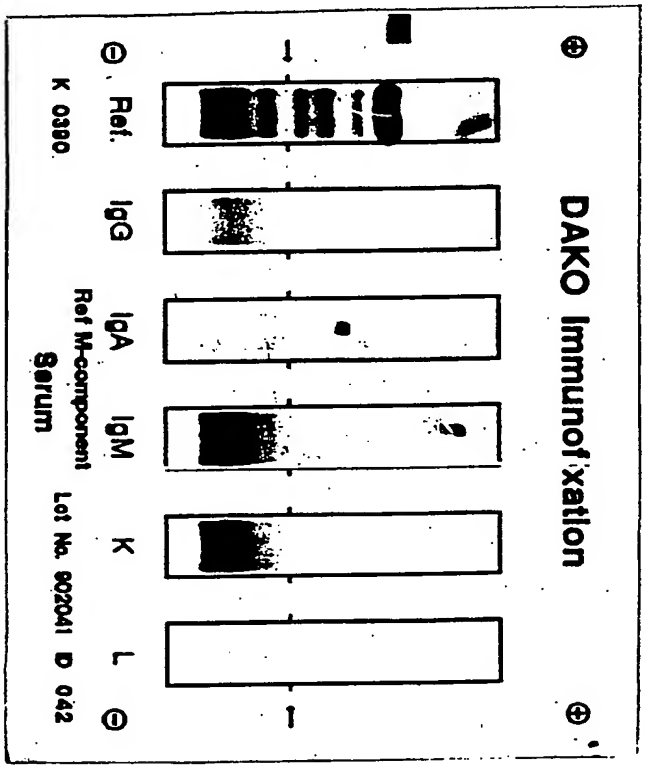
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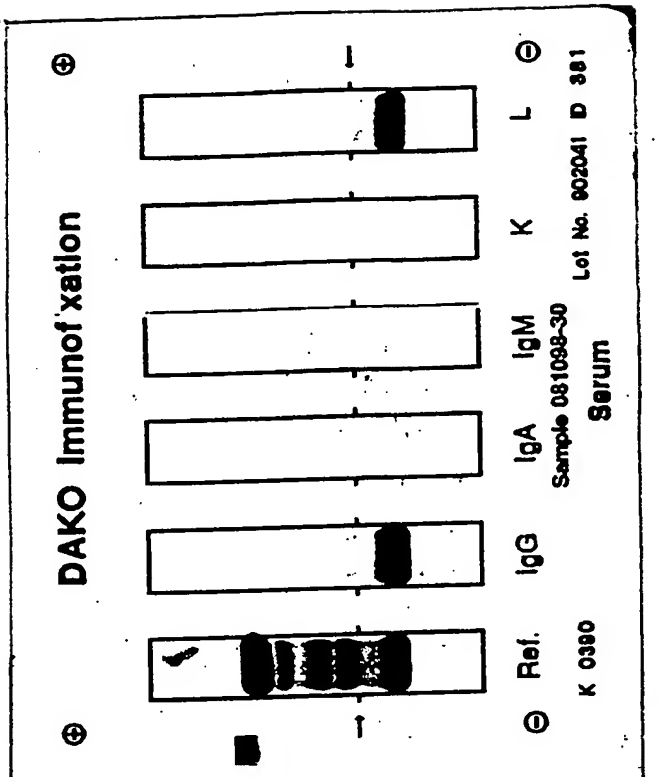
Fig. 2B

Fig. 2D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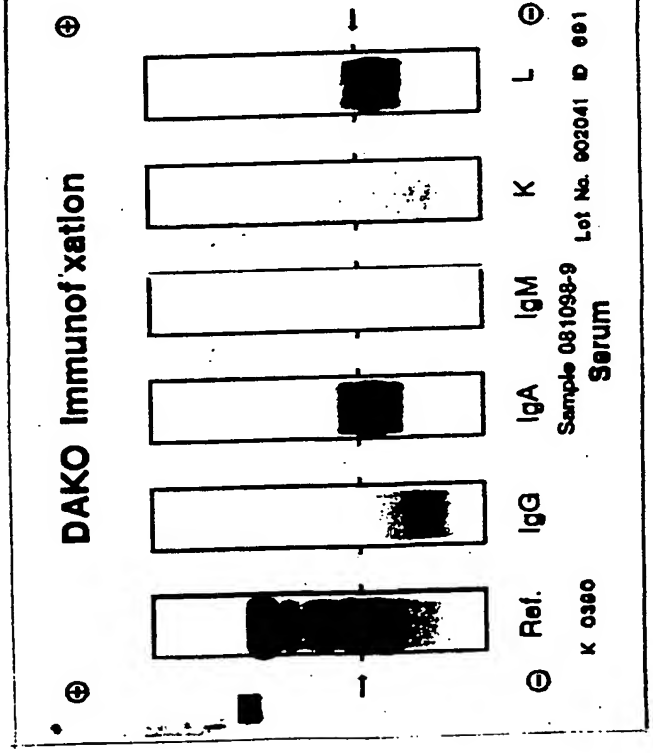
K 0380

Fig. 2C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IgM
K
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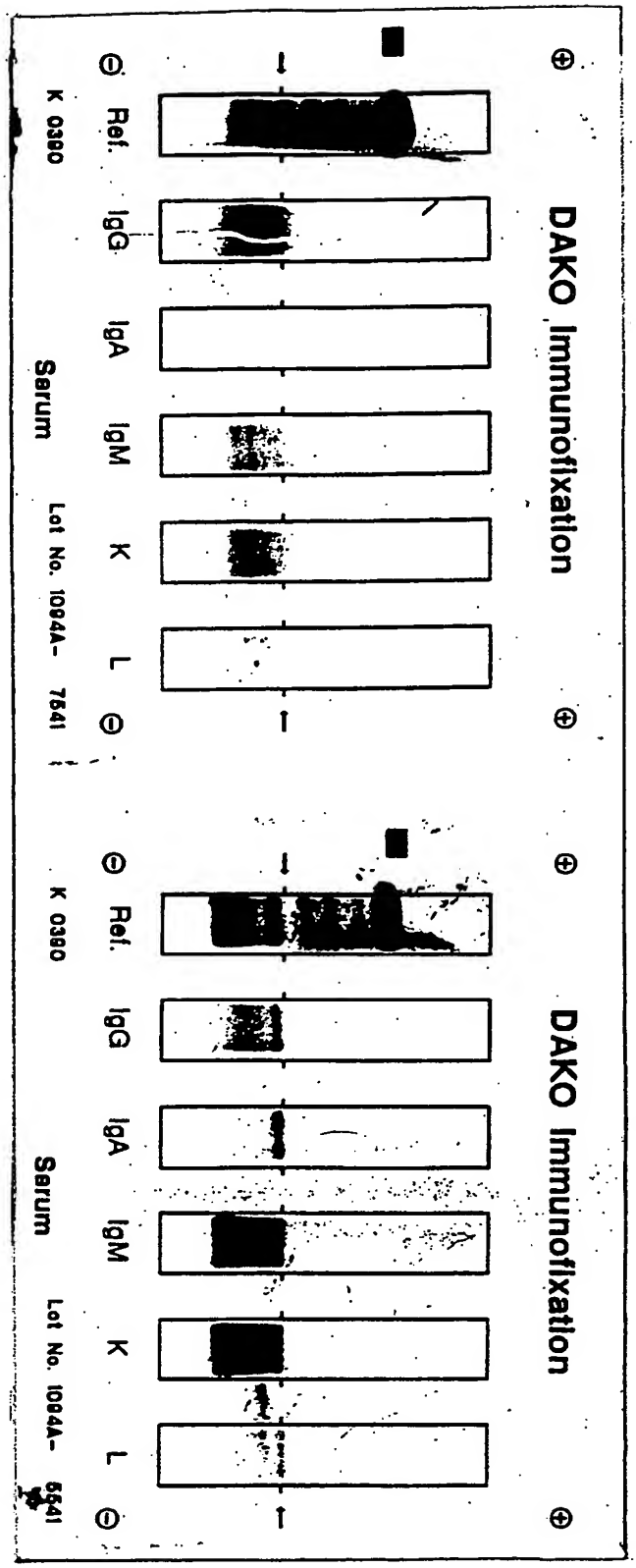
Lot No. 902041 D 681
K 0380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K 0390
Serum
Lot No. 1094A- 7541
DAKO Immunofixation
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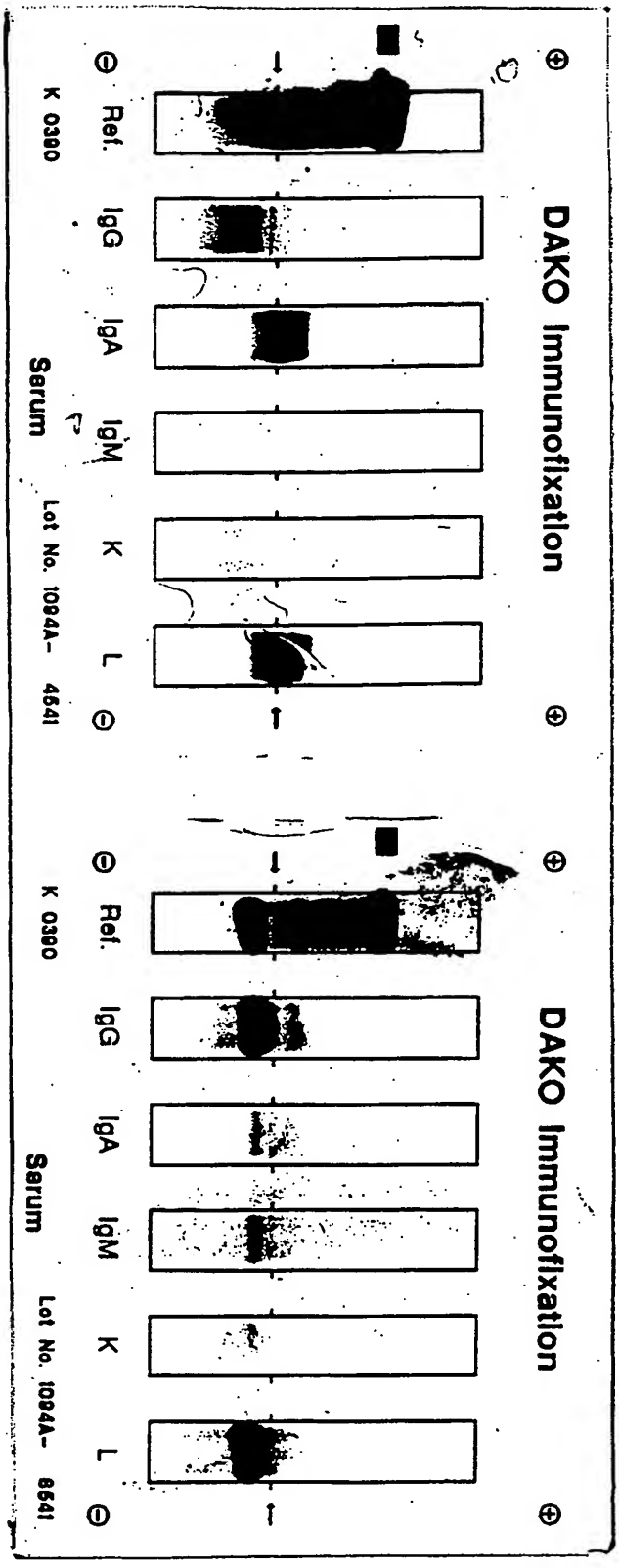
K 0390
Serum
Lot No. 1094A- 6541

Fig. 3A

Fig. 3B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DAKO Immunofixation
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K 0390
Serum
Lot No. 1094A- 8541

Fig. 3C

Fig. 3D

INTERNATIONAL SEARCH REPORT

Inte ›nal Application No
PCT/DK 00/00350

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/561 G01N33/48 G01N33/68 G01N27/447 B01D57/02
G01N33/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, CHEM ABS Data, MEDLINE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TARNAI, M. ET AL: "Capillary electrophoretic separation of mono- and dimaltosyl-.beta.-cyclodextrins and determination of the stability constants of their benzoate complexes." CHROMATOGRAPHIA, vol. 48, no. 5-6, 1998, pages 383-387, XP000856531 cited in the application page 384, column 1, paragraph 4; figures 1-4 --- -/--	1-22

[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 November 2000	24/11/2000

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1

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/DK 00/00350

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	TAWALI, A. B. ET AL: "Determination of phytic acid content of soya beans during tempeh production using capillary electrophoresis." DEUTSCHE LEBENSMITTEL-RUNDSCHAU, vol. 94, no. 1, 1998, pages 28-30, XP000856532 cited in the application page 28, column 2 ---	1-22
X	AHUMADA, I. ET AL: "Determination of organic acids and phosphate in soil aqueous extracts by capillary zone electrophoresis." COMMUNICATIONS IN SOIL SCIENCE AND PLANT ANALYSIS, vol. 30, no. 1-2, 1999, pages 213-220, XP000856533 cited in the application abstract ---	1-22
X	ARELLANO, M. ET AL: "Capillary electrophoresis and indirect UV detection as a fast and simple analytical tool for bacterial taxonomy." J. CHROMATOGR., A (PRESENTED AT THE 9TH INTERNATIONAL SYMPOSIUM ON HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS HELD IN ANAHEIM, CA, USA, 26-30 JAN 1997), vol. 781, no. 1-2, 1997, pages 497-501, XP004094582 cited in the application figure 2 ---	1-22
X	LUNA, E. A. ET AL: "Evaluation of the utility of capillary electrophoresis for the analysis of sulfobutyl ether.beta.-cyclodextrin mixtures" JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS, vol. 15, no. 1, 1996, pages 63-71, XP000856460 cited in the application abstract ---	1-22
X	CHANKVETADZE, BEZHAN ET AL: "Capillary electrophoresis enantioseparation of noncharged and anionic chiral compounds using anionic cyclodextrin derivatives as chiral selectors" JOURNAL OF CAPILLARY ELECTROPHORESIS, vol. 2, no. 5, 1995, pages 235-240, XP000856503 cited in the application abstract; figures 1,2 ---	1-22

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/DK 00/00350

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 891 422 A (CARLIN EDWARD ET AL) 6 April 1999 (1999-04-06) column 8, line 36 - line 36; claim 1	1-10,22
A	US 4 321 119 A (AMBLER JEFFREY) 23 March 1982 (1982-03-23) cited in the application the whole document	1-22

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte[illegible]nal Application No
PCT/DK 00/00350

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5891422 A	06-04-1999	NONE	
US 4321119 A	23-03-1982	AU 6189780 A	14-05-1981
		EP 0034233 A	26-08-1981
		JP 56076040 A	23-06-1981

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